

Effect of Moderate Intensity Exercise on Body Weight and Blood Estrogen Level Ovariectomized Mice

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Abstract

Estrogen is a hormone which is related to lipid oxidation, lipogenesis inhibition, and lipolysis rate. The purpose of this study was to determine the effect of moderate intensity exercise on body weight and blood estrogen level in ovariectomized mice (ovx). The study was held in the Laboratory of Biochemistry, Faculty of Medicine Universitas Airlangga during the period of April to May 2017 on old nineteen 3-4 months old female mice, 20-30 grams of weight, which were divided into two groups: OVX (n=9, ovx without exercise) and OVX+E (n=10, ovx and moderate intensity exercise). Moderate intensity exercise in this study included swimming, three times a week at 3 pm for four weeks using a 6% of body weight load. Experiment data were analyzed by paired t-test and independent t-test using SPSS. The average body weights of the OVX group were 26.44 ± 1.94 grams and 29.89 ± 2.62 gram at pretest and posttest, respectively, while for the OVX+E group, the weights were 26.50 ± 2.59 grams and 26.40 ± 2.07 grams. There were asignificant intergroup body weight differences (Δ OVX= 3.44 ± 2.55 gram and Δ OVX+E= -1.00 ± 1.37 gram) with $p=0.004$. Blood estrogen level in OVX+E (12.04 ± 10.13 ng/ml) was higher than in control (OVX= 11.24 ± 8.33 ng/ml); however, the difference was not significant ($p=0.855$). Moderate intensity exercise reduces body weight, without any significant increase in blood estrogen level. Hence, the body weight loss is not caused by estrogen, which may be due to the short period of exercise in this study that the estrogen has not had the time to increase.

Key words: Blood estrogen level, exercise, moderate intensity, ovariectomy, obesity

Pengaruh Olahraga Intensitas Sedang terhadap Berat Badan dan Kadar Estrogen Darah pada Mencit Ovariektomi

Abstrak

Estrogen merupakan hormon yang berperan pada oksidasi lipid, kecepatan lipolisis, dan hambatan lipogenesis. Penelitian bertujuan mengetahui pengaruh olahraga intensitas sedang terhadap berat badan dan kadar estrogen darah pada mencit ovariektomi (ovx). Penelitian dilaksanakan di Laboratorium Biokimia Fakultas Kedokteran Universitas Airlangga, April-Mei 2017. Sejumlah 19 mencit betina berusia 3-4 bulan, berat 20-30 gram, dibagi menjadi dua kelompok: OVX (n=9, ovx tanpa perlakuan) dan OVX+E (n=10, ovx dengan olahraga intensitas sedang). Olahraga intensitas sedang dilakukan dengan renang, tiga kali seminggu pukul 15.00 WIB selama empat minggu dengan beban 6% dari berat badan. Data dianalisis dengan *paired t-test* dan *independent t-test* SPSS. Berat badan OVX pra-tes $26,44 \pm 1,94$ gram dan pascates $29,89 \pm 2,62$ gram. Berat badan OVX+E pra-tes $26,50 \pm 2,59$ gram dan pascates $26,40 \pm 2,07$. Terdapat perbedaan bermakna berat badan antarkelompok (Δ OVX= $3,44 \pm 2,55$ gram and Δ OVX+E= $-1,00 \pm 1,37$ gram), $p=0,004$. Kadar estrogen darah OVX+E ($12,04 \pm 10,13$ ng/mL) lebih tinggi daripada kontrol (OVX= $11,24 \pm 8,33$ ng/mL), tetapi tidak berbeda bermakna ($p=0,855$). Olahraga intensitas sedang menurunkan berat badan, tetapi tidak bermakna meningkatkan kadar estrogen darah. Penurunan berat badan tidak disebabkan oleh estrogen. Peningkatan estrogen belum terjadi mungkin disebabkan oleh waktu yang kurang lama.

Kata kunci: Kadar estrogen darah, obesitas, olahraga intensitas sedang, ovariektomi

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Introduction

Overweight and obese Indonesian population over 18 years old increased from year to year. It's prevalence in 2013 has reached 28.9% of the adult population.⁴ Obesity is found in postmenopausal women.¹ Visceral fat is increased by 30% in postmenopausal women and related to cardiovascular disease, insulin resistance, and cancer.⁵ Women's obesity often occurs due to low blood estrogen levels⁶ and high body weight.⁷ Estrogen and progesterone hormone therapy as a solution and prevention of postmenopausal women's obesity increases breast cancer risk,⁸ so a suitable strategy to prevent obesity of postmenopausal women is needed. In addition to increasing energy output, physical activity can also increase the expression of genes that encode aromatase. Aromatase is an enzyme that plays a role in estrogen hormone synthesis.⁹ Estrogen decreases visceral adipose tissue by reducing the expression of SREBP and lipoprotein lipase which play a role in lipogenesis³.

Swimming with moderate intensity is a good intervention for postmenopausal women, it doesn't have a significant negative impact on the body,¹⁰ but its effects on estrogen hormone and body weight in postmenopausal women remain unknown clearly. Temporary suspicion is that moderate intensity of swimming affects blood estrogen level and body weight so it can be used as a preliminary strategy to prevent obesity. The purpose of this study was to determine effect of moderate intensity exercise on body weight and blood estrogen level of ovariectomized mice (ovx).

Methods

The study was conducted by posttest only control group design approach under the approval of Medical Research Ethics Committee Faculty of Medicine, Universitas Airlangga Surabaya (188/EC/EPK/FKUA/2016). Twenty-six mice (BALB/c) 3–4 mo aged, 20–30 gram were obtained from Animal Models Laboratory of Biochemistry Department, Faculty of Medicine Universitas Airlangga. The study has been held on April–May 2017. Animals were maintained in some cages in the room temperature 25–30°C, with enough food and drinking water.

Mice were excised by dorsal incision with general anesthesia which are obtained by mixing ketamine 0.5 mL and xylazine 0.5 mL in 1 mL syringe, then intraperitoneal injection was done

on mouse 0.05 mL each. Once anesthetized, the abdominal incision began, the fallopian tube were ligated, and the ovaries were cut and discarded. Open wounds due to incisions were covered with a suture procedure. Mice were given seven days to recover.

The 19 mice were acclimatized for a week and then divided into two groups, which are control group (OVX) and exercise group (OVX+E). In the control group, mice were not given by Moderate Intensity Exercise (MIE) intervention. In other hand, MIE (three times a week for four weeks) was performed to exercise group every 15.00 WIB with load 6% of body weight that was done by calculating the maximum duration of swimming ability each mouse. Duration of exercise was taken from 80% of the maximum time achieved by each mouse.

Animal weights were measured in all groups, at four days before ovariectomy, just before ovariectomy, a week after ovariectomy, and every week during exercise using a torbal (torsion balance) scales Camry EK3250 in gram using with precision one digit behind the comma.

In the end of experiment, mice were eventually terminated and their blood samples were taken from the heart. Then blood estrogen level was measured using ELISA method Sigma Aldrich in pg/mL.

Data were analyzed for distribution normality with Kolmogorov Smirnov test and different test using independent t-test. Comparison between body weight before and after exercise were analyzed using paired t test. Data analysis using SPSS version 17.

Results

Initial weight until a week after ovariectomy done in each of two groups can be seen in table 1. The measurement of BW initial (body weight four days before ovariectomy), BW pre-OVX (body weight just before ovariectomy), and BW post-OVX (body weight a week after ovariectomy) were homogenous statistically and data were in normal distribution (Table 1). All mice in both control and exercise group 3 mo aged.

After mice were ovariectomized and performed by swimming according to each group, there is a body weight gain in the negative control group, while in exercise group the reduction of body weight was eventually shown.

Figure 1 shows the MIE has the potency in preventing the body weight gain of ovariectomized mice. Body weight OVX (blue

Table 1 Body Weight of Mice Before Exercise

Group	n	Body Weight Before Exercise (gram)	Independent t Test
OVX	9	26.44 ± 1.94	Homogeneity =0.415 p=0.959
OVX + E	10	26.50 ± 2.59	

*OVX is ovariectomized mice without exercise

Table 2 Blood Estrogen Level After Four Weeks Exercise

Group	N	Blood Estrogen Level (ng/mL)			Independent t Test
		Mean ± SD	Min	Max	
OVX	9	11.24 ± 8.33	1.04	19.93	Homogeneity= 0.014; F= 7.439; p= 0.855
OVX + E	10	12.04 ±10.13	1.46	22.87	

*OVX is ovariectomized mice without exercise; ** OVX+E is ovariectomized mice moderate intensity exercise

line) in a first week raise up, then goes down in the second week, but in third week until end of experiment it shows an increasing line again (body weight has significant difference compare to initial point, $p= 0.004$). In the other side, body weight OVX+E (orange line) fall until second week, then increase again in the third week till end of experiment without significant difference compare to initial point ($p=0.823$) *significant difference was noted ($p<0.05$) between the control group and the exercise group.

Estrogen level was measured from the blood that was obtained from the mouse heart.

Before taking the blood, intervention of mice in exercise group was stopped to avoid the acute effect of exercise. The result of blood estrogen level in both groups can be shown in table 2. The measurement of blood estrogen level wasn't homogeny statistically but data was in normal distribution.

The level of estrogen was relatively the same and there is no significant difference between two groups ($p=0.855$) although the level in exercise group (12.04 ± 10.13 ng/mL) was higher than control (11.24 ± 8.33).

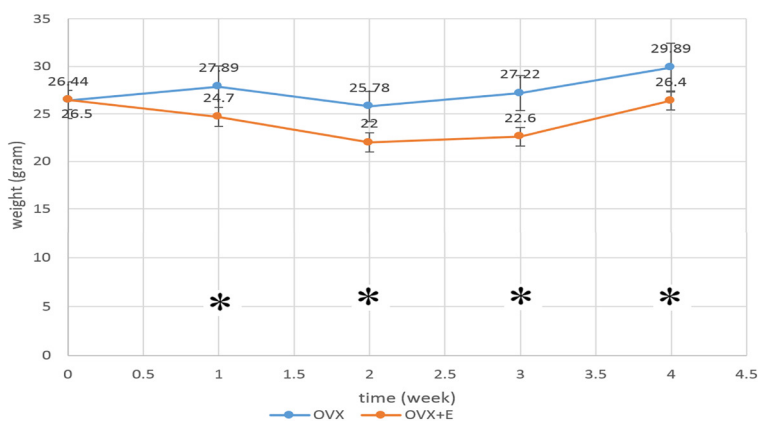


Figure 1 The MIE Has the Potency in Preventing the Body Weight Gain of Ovariectomized Mice

*OVX is ovariectomized mice without exercise; ** OVX+E is ovariectomized mice moderate intensity exercise

Discussion

Female mice was chosen to perform ovariectomy which had an intention to represent postmenopausal condition in woman and easily evaluate hormone level as the effect of exercise only (without involvement of estrogen production from ovary). The range of 3–4 mo age was chosen because this range is adult age for mice as the increasing prevalence of obesity in woman.

The physical properties of swimming are good for postmenopausal women because they do not negatively affect bone¹¹ and muscle.¹⁰ Compared to treadmill running and spontaneous wheel exercise, the advantage of swimming is not causing foot injuries, so it may be less physically traumatic to the animal. The Uniform type of physical activity can be found in swimming when applied in appropriate way, there is no “stop and go” activity as that found in treadmill running.

Continuous swimming (mild, moderate to heavy exercise when weights are attached to their tails) produces significant recruitment of both the forelimb and hindlimb muscles, although the pattern of recruitment may differ from that which occurs with treadmill running, based on reduction of glycogen and skeletal muscle blood flow studies.¹²

Moderate intensity exercise using load attached to mice tail has function to set the exercise intensity which result in heart rate as same as human heart rate with the same type of intensity. Body weight is the easiest macro indicator used to evaluate the balance of energy inside our body. The weight gain is found in the negative control with the significant different ($p < 0.05$).

This finding support the previous research which has successfully proven that exercise would prevent a weight gain in moderate intensity exercise group ovariectomized mice.¹³ Lower weight gain in exercise group than control group also found in result study of 6 weeks moderate intensity exercise ovariectomized mice.¹⁴

Other finding, that were performed MIE had higher blood estrogen level than control, but analysis independent t-test showed no significant difference ($p > 0.05$). These results support the results of the Bebasari which showed no significant difference serum estrogen levels in ovariectomized rats between control and treadmill group for 8 weeks with frequency 5 times a week.¹⁵ In the study of Hao et al.¹⁶ there was a significant increase in serum estrogen

levels after treadmill ovariectomized rats compared with ovariectomized rats without intervention.

Different results are caused by differences in exercise type, duration of exercise, and frequency. Research of Hao et al.¹⁶ used treadmill or running exercise with the same duration from start to finish that was 60 minutes. Treadmill exercise was done in 12 weeks with frequency five times a week. Longer treatment is required more than Bebasari¹⁵ study or approximately 12 weeks as duration as in Hao et al.¹⁶ study to make a significant increase blood estrogen levels.

Moderate intensity exercise can prevent weight gain on ovariectomized adult mice. There is no significant difference blood estrogen level between control and exercise group although blood estrogen level exercise group higher than control. Body weight loss in ovariectomized mice was not caused by involvement blood estrogen level for four weeks moderate intensity exercise intervention.

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